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Comparison of the PT/INR assay using two different reagents

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Abstract

Aim: The Prothrombin time (PT) assay is a coagulation test that shows the activation of the extrinsic and final common pathways. The purpose of the present study was to evaluate the PT/INR levels using BioMedica QuikCoag PT and Technoclone Technoplastin HIS (heparin-insensitive) reagents on Ceveron alpha coagulation instrument.

Materials and Methods: The study was conducted in the Usak State Hospital in the west of Turkey. We analyzed 78 patients plasma PT/INR levels utilizing two kits on Ceveron alpha automated coagulation instrument.

Results: The mean age of 32 male 46 female patients was 51 ± 21. The mean value of PT was 14,77 ± 5,80 with Technoplastin HIS and 20,66 ± 12,19 with QuikCoag PT. The mean value of INR was 1,42 ± 0,65 with Technoplastin HIS and 1,67 ± 1,16 with QuikCoag PT. We obtained a statistically significant difference between PT and INR levels using a two-sample t-test (p=0,000 and p=0,000 respectively).

Discussion: Technoplastin HIS PT is a standardized Ca-thromboplastin reagent obtained from the rabbit brain. The values attained utilizing Quik Coag PT kits are not equal to those attained utilizing the Technoplastin HIS PT kits. An independent check of a producer's international sensitivity index (ISI) assignments by a national reference laboratory in Turkey is also proposed.

Keywords

Prothrombin Time; INR; Thromboplastin; Reagent

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Introduction

The prothrombin time (PT) assay is a coagulation test that shows the activation of the extrinsic and final common pathways. Extrinsic coagulation starts with tissue factor and finalizes in fibrin formation. It is now broadly used to screen for abnormal coagulation and monitor for oral anticoagulant therapy (OAT) with warfarin potassium. However, test results can be conflicting between laboratories because thromboplastin reagents used in PT measurement can vary and is affected by the species-tissue used to manufacture it as well as the applied method. To solve the problem and to standardize results, the World Health Organization suggested that the PT ratio measured using local thromboplastin should be transformed into the international normalized ratio (INR) scale using human brain thromboplastin as the international normalized sample. The PT/INR is now measured by laboratories at many medical institutions.

Expert committee on biological standardization of the World Health Organization has described the INR with the formula below:

$INR=(PT\ patient\ /MNPT)^{ISI}$

PT patient: the PT for patient's blood

MNPT: the geometric mean of prothrombin time (MNPT) of the healthy adult population

ISI: The international sensitivity index of the set up [1].

External quality program reports show that there is a demand for advancement in the between laboratories INR alteration [2,3,4].

An INR level of 1 is seen as healthy. Although an actual goal of INR level for any medical situation can be somewhat altered, an all over goal INR level of 2,5 is advised for most medical situations needing OAT [5].

Therefore, determinations of INR are frequently implemented in different areas by use of multiple types of devices, it is significant that the variations in measurements between instruments are reduced and that the reasons of these divergences are assumed. Settlement in results among laboratory PT/INR devices and methods healed afterward the promotion of WHO guidelines for thromboplastins and plasma used to control oral anticoagulant therapy (1999), that initiated new calibration procedures. Though, concordance of measurements from various laboratories endures stumping [6,7,8]. Various groups and sensitivities of thromboplastins and reciprocal actions among thromboplastins and coagulation factors of individual patients may affect the certainty of the devices [7,9,10].

In the present work, two distinct reagents were analyzed on the same analyzer in the same samples. The two reagents selected both have low ISI values. The Quick Coag PT reagents is a lyophilized preparation of human tissue recombinant thromboplastin. Technoplastin HIS PT is a standardized Ca-thromboplastin reagent obtained from the rabbit brain.

The purpose of our study was to evaluate the PT and INR levels utilizing BioMedica Quik Coag PT and Technoclone Technoplastin HIS (heparininsensitive) reagents on Ceveron alpha coagulation instrument (Austria) in the same specimens.

Material and Methods

The present work was established in the multi-specialty Usak State Hospital in the west of Turkey. All procedures of the study were approved by the Usak University of Medical Faculty Ethics Committee. We evaluated 78 patients' plasma PT and INR levels using Biomedica Quik Coag PT Reagent (Biomedica Diagnostics Inc, Windsor, Canada) and Technoclone Technoplastin HIS PT Reagent (Technoclone GmbH, Vienna, Austria) commercial reagents on Ceveron alpha automated coagulation instrument (Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Vienna, Austria). The mean age of 32 male 46 female patients was 51 ± 21 years. Venous blood specimens (2,7 mL) were at the same time obtained from all participants with BD vacutainer tubes containing 0,109M sodium citrate and centrifuged at 2.500xg for 15 minutes. The PT/INR was detected as soon as in the central laboratory by utilizing both reagents sequentially on Ceveron alpha coagulation instrument on the same day.

All plasma samples were collected from patients who were admitted to the cardiology clinic. Specimens with clot and inadequate samples were excluded from the study. Tests on fresh plasma specimens were measured within 2 hours after collection.

The Procedure with BioMedica Quik Coag PT Reagent is described below.

Reconstituted Quik Coag PT reagent pre-incubated to 37°C for at least 10 minutes by a laboratory technician. The suspension of the reagent was maintained by magnetic stirring immediately prior to use. The next steps were performed on the Ceveron alpha coagulation analyzer. The test plasma (50 µL) was pipetted into a test cuvette and incubated at 37°C for 1 minute. Rapidly 100 µL of the pre-incubated Quik Coag PT reagent was added, simultaneously the timer was started. Finally, clotting time was recorded in seconds.

The procedure with Technoclone Technoplastin HIS PT reagent is is described below.The lyophilized reagent is reconstituted with distilled water at room temperature by the same laboratory technican. The next steps were performed on the Ceveron alpha coagulation analyzer. The same citrated plasma (0,1 mL) was pipetted into a test cuvette and incubated at 37°C for 1 minute. Then 0,2 mL of Technoplastin HIS PT reagent was added. The clotting time was determined in seconds.

Statistical Analysis

Statistical analysis was performed using SPSS version 20. Data from the BioMedica and Technoclone reagents were evaluated utilizing the two-sample t-test. P- value <0,05 was noted to demonstrate statistical significance.

Results

A total of 78 patients had the mean age of 51±21 years (32 male, 46 female). Table 1 shows mean, median, range, SD and p-values for PT and INR test obtained using Quick Coag PT(ISI:1,01) and Technoplastin HIS PT (ISI:1,08) reagents. We obtained 28,5 % and 14,97 % higher levels of PT and INR results with Quick Coag PT than Technoplastin HIS PT. We showed a statistically significant difference between PT and INR levels using a two-sample t-test (p=0,000 and p=0,000 respectively).

Linear regression analyses demonstrated a good correlation between both reagents for PT and INR values (r=0,977 and r=0,942 respectively)(Figure 1 and Figure 2).

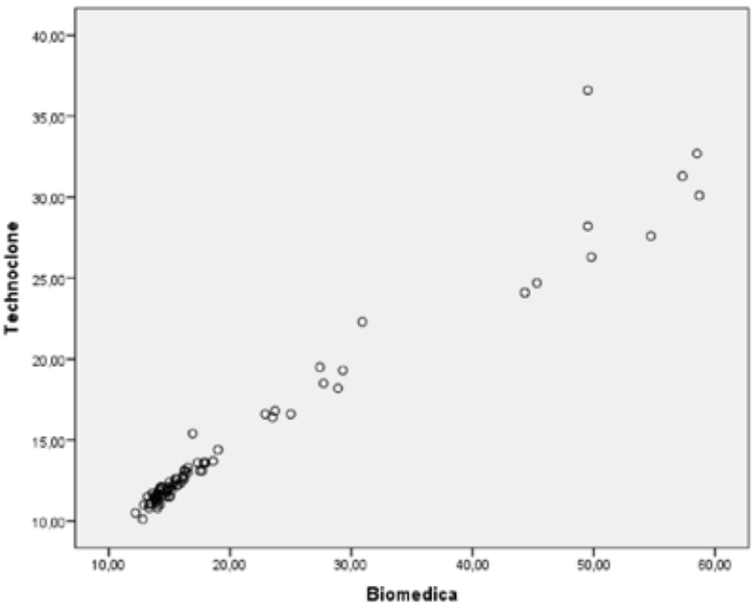

Figure 1. Linear Regression analysis for PT results.

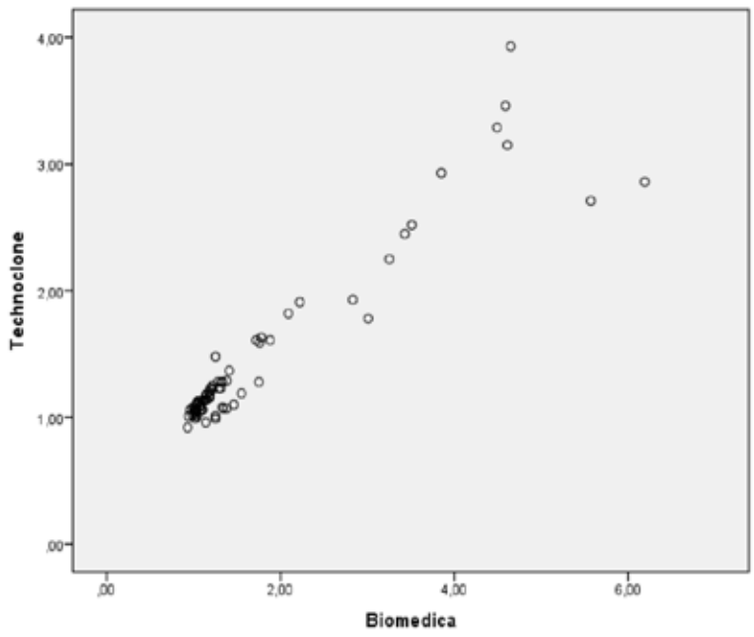

Figure 2. Linear Regression analysis for INR results.

Table 1. p value between Technoplastin HIS and QuikCoag PT ragents.

Test Name	Parameters	Technoplastin HIS	QuikCoag PT	P value
PT	Mean	14,77 s	20,66 s	0,000*
	Median	12,30	15,45	
	Range (min-max)	10,10-36,60	12,20-58,70	
	SD	5,80	12,19	
INR	Mean	1,42	1,67	0,000*
	Median	1,15	1,19	
	Range (min-max)	0,92-3,93	0,93-6,19	
	SD	0,65	1,16	

PT: prothrombin time, INR: international normalized ratio, SD: standart deviation.

Discussion

Horsti et al. obtained plasma specimens from 150 patients for whom oral anticoagulants had been assigned. PT was measured by utilizing seven marketable reagents and four calibrator groups. They found that the mean measurements altered significantly (p<0,001) for 17 of 21 available coupled associations of methods. Only two couples of methods yielded very close measurements. They concluded the settlement between certain available INR methods is inferior in quality [6]. Our results are similar to Horsti et al. The results of Technoplastin HIS PT reagent are significantly lower (p=0,000).

The study from North America evaluated a recombinant thromboplastin on four different analyzers. They concluded that ISIs of the same reagent differ significantly on distinct four analyzers. They also offer a new procedure using local normal donors, locally warfarin pooled plasmas, and certified plasma set [11].

A novel prothrombin reagent which uses human recombinant tissue was compared with two known reagents. The research showed refined and equivalent performance for the new reagent. Two recombinant reagents caused less interference from lupus anticoagulant than placental thromboplastin [12].

The measurements attained using Quick Coag PT reagents are not comparable to those attained using the Technoplastin HIS PT reagent. The most probable cause of higher values of PT/INR test measurements with Quick Coag PT than Technoplastin HIS PT may be the use of human tissue recombinant thromboplastin. The results of Quick Coag PT were more consistent with patients' clinical situations.

ISI calibration for the regional analysis combination, the real results of the certified plasma PT will vary conforming to the types of the standard thromboplastin utilized, and thus PT results must be freely certified for the various types. Since the direct INR detection advances, plasma INR results should hypothetically be equal whichever reference thromboplastin kit is utilized. In reality, variation in INRs measured using diverse thromboplastins has been monitored with some frozen dried plasmas; levels should not be centered into an individual INR if the INRs measured with particular general kits vary by more than 10% of the mean. Enormous inconsistency among INRs measured with distinct thromboplastins can demonstrate that the plasmas are inappropriate for utilize with thromboplastins of all species. It should be considered that the producer or distributor of the certified plasmas should obviously determine the pair of kit-device compositions with which their supplies can be faithfully utilized [13].

The INR arrangement for PT setting up was essentially considered on manual detection of PTs, and pictured the determination of an indivigual ISI level for a various group of thromboplastin kit [14]. Nevertheless, recently the manual PT has been nearly globally changed by coagulometer devices, and a lot of researches have represented that the ISI of thromboplastin kits alters with respect to the sort of device utilized [15,18]. A number of producers have presented special ISIs, but this does not solve the issue absolutely due to a number of available device-kit compositions and since ISIs frequently alter with the equal thromboplastin even between devices of the same kind. Regional PT combination (i.e. thromboplastin-coagulometer set up) ISI calibration thus looks crucial while standard evalu-

ation of the INR with certified plasmas demonstrates inferior achievement. ISI calibration utilizing the WHO advised protocol is not generally achievable in general hospital laboratories for a diversity of causes, involving the necessity for manual PT detecting with an accepted thromboplastin arrangement. WHO thromboplastin standards or secondary thromboplastin standards are not easily applicable to general hospital laboratories. Moreover, the WHO protocol demands a specimen of 20 fresh blood from healthy subjects and 60 fresh blood from fixed oral anticoagulated individuals. The need for a huge number of fresh blood is a notable restriction on the achievement of thromboplastin ISI calibrations in a lot of laboratories [19].

The divergence is because of calibration errors that continue due to the distinct International Reference Preparations were not controlled versus each other in the real researches. A current protocol has now been decided about International Thromboplastin Reference Preparations, so what their source and constitution, will be calibrated versus total available International Reference Preparations for to assure harmony of results between different lines of calibration [20]. It is advised by the WHO Expert Committee on Biological Standardization that the International Reference Preparation of the same types or combination should be used for calibration of secondary standards, e.g. handling standards, by producers and national reference laboratories.

The extensive utilization of these International Reference Preparations for the calibration of secondary standards shows the worth put on them by the scientific community in charge of the check of thromboplastins. A free check of a producer's ISI assignments by a national reference laboratory is also proposed. National inspect authorities should think to describe a professional laboratory in their country for controlling thromboplastin kits and plasmas used by medical laboratories to check oral anticoagulant treatment to provide that they are in concordance with guidelines announced by WHO.

In conclusion, we tested two different reagents for PT/INR using 78 patients' samples on the same analyzer. A comparison of the PT and INR test results showed a clinically disagreeable difference between two different reagents. When PT results are reported, INR (14,97%) should be given together with PT (28,5 %) value since there are fewer variations as we have shown. It can be useful to assign local ISI specific to reagent/instrument using with local MNPT and locally warfarin pooled plasmas. Better approaches are needed.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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